

## Introduction and background

Several hundred chemicals have been identified as carcinogens by scientific agencies, yet many thousands of chemicals have never been evaluated for carcinogenic potential. Chemical and toxicogenomic databases are promising resources for quickly identifying and prioritizing bioactive environmental chemicals.

Using chemical activity data from the US EPA Toxicity Forecaster's (ToxCast's<sup>TM</sup>) ACEA, Apre dica, and BioSeek platforms, the Comparative Toxicogenomics Database<sup>TM</sup> (CTD), and expert judgement, we developed a novel screening approach to identify and rank chemicals active in assays we considered to be cancer pathway-related. For the top 50% of ToxCast chemicals most active in these cancer pathway-related assays, we examined the primary commercial/industrial uses. We also determined which chemotypes (generic structural fragments) are enriched in the top 5% most active chemicals.

## Methods

*Assay and chemical selection from US EPA's ToxCast program*

We screened a total of 236 assays in the US EPA Toxicity Forecaster's (ToxCast's<sup>TM</sup>) ACEA, Apre dica, and BioSeek platforms. Of these, we identified 100 assays to be related to cancer pathways, based on expert judgement (n = 30) and by consulting the Comparative Toxicogenomics Database<sup>TM</sup> (CTD) (n = 70). The CTD is maintained by the North Carolina State University's NIEHS Environmental Health Science Center. The 30 cancer pathway-related assays identified by expert judgement included assays evaluating increased cell proliferation and other assays associated with cancer based on evidence from the scientific literature (e.g., protein modifications associated with genotoxicity). The 70 cancer pathway-related assays identified using the CTD were in the BioSeek platform and interrogated protein targets. Each of these protein targets had at least 3 curated associations with cancer in the CTD (December 8, 2015 version). We included assays that evaluated both increased and decreased expression of these protein targets. Cancer pathway-related assay selection is diagrammed in Figure 1.

Activity data for 1,061 chemicals from Phases 1 and 2 of ToxCast were exported from the Interactive Chemical Safety for Sustainability (iCSS) ToxCast Dashboard on November 30, 2015. Of these, 984 had been tested in all 100 cancer pathway-related assays and thus were the chemicals we examined in this work.

*Qualitative evaluation of the biological coverage of cancer pathway-related assays*

We mapped each of the 100 cancer pathway-related assays to the 10 key characteristics of carcinogens described by the International Agency for Research on Cancer (IARC, 2015), in order to qualitatively evaluate biological coverage. Some of the assays were mapped to 2 characteristics. We also compared our mapping results for assays from 3 ToxCast platforms with IARC's independent mapping of 274 cancer pathway-related assays they selected from all 7 ToxCast platforms. This information is displayed in Table 1.

*Ranking of chemical activity in cancer pathway-related assays*

The concentration inducing a half-maximal response (AC<sub>50</sub>) was used for each active chemical-assay pair, and an arbitrary value of "1,000" was used for each inactive chemical-assay pair. The AC<sub>50</sub> and inactive values for each of the 984 chemicals in the 100 cancer pathway-related assays were summed to generate an overall activity score, and the chemicals were ranked by that activity score in order from lowest to highest (with lowest being most active). The activity score thus captures both chemical potency and the quantity of assays in which a chemical was active. The use categories of the 50% of chemicals (n = 372) most active in the cancer pathway-related assays are shown in Figure 2. The use category data were adapted from the information available on the iCSS ToxCast Dashboard. The 5% (n = 50) of chemicals most active in the cancer pathway-related assays are listed in Table 2. Table 2 also notes which chemicals are listed as known to the state to cause cancer under California's Proposition 65 (Title 27, California Code of Regulations, § 27001).

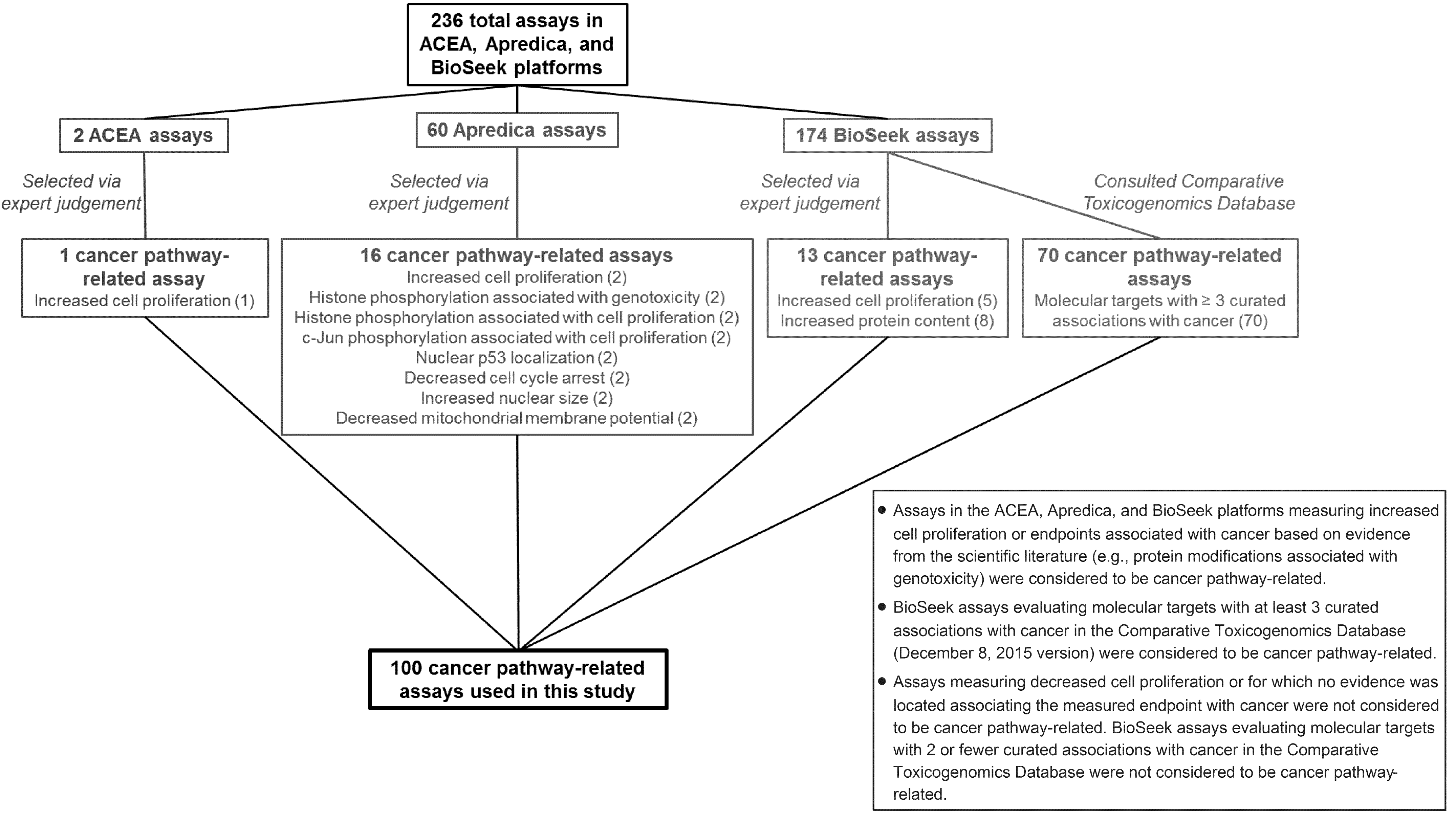
*Chemotype evaluation*

The ChemoTyper application, developed by Molecular Networks GmbH, and the "TOXCST\_v4a\_1892\_20Mar2012.sdf" and "toxprint\_v2.0\_r212.xml" files were used for our chemotype evaluation. We determined the number of chemotypes represented in the 5% (n = 50) of chemicals most active in the cancer pathway-related assays by searching across "any" of them in the application, including any Ashby-Tennant structural alerts for DNA reactivity and cancer threshold of toxicological concern (TTC) structures.

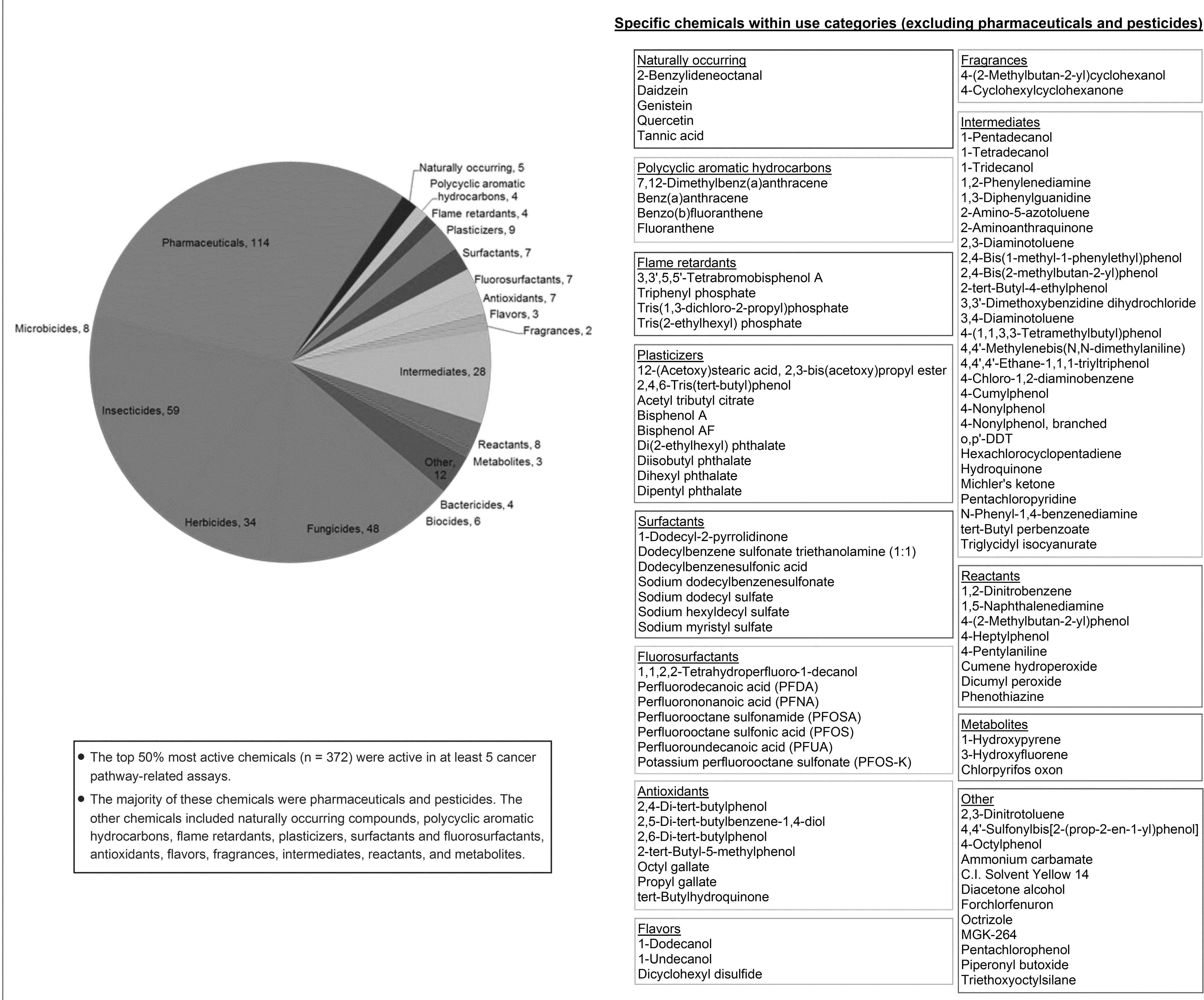
One-tailed two-proportion Z-tests were conducted using the Bonferroni correction to compare the proportion of each chemotype (n = 194) in the 50 most active chemicals with the proportion in the remaining 934 chemicals. The null hypothesis was that the proportion of each chemotype in the 50 most active chemicals was not different from the proportion of each chemotype in the remainder of the chemicals; the null was rejected when p < 2.6x10<sup>-4</sup>. This allowed us to determine which chemotypes are enriched in the 5% of chemicals most active in the cancer pathway-related assays.

## Results

**Figure 1.** Selection of cancer pathway-related assays.



**Figure 2.** Use categories of the 50% of chemicals most active in cancer-pathway related assays.



**Table 1.** Biological coverage of key characteristics of carcinogens in selected cancer pathway-related assay endpoints.

Key characteristic of carcinogens	Percentage of cancer pathway-related assays in a subset of ToxCast platforms (ACEA, Apre dica, and BioSeek) linked to characteristic <sup>a</sup>	Percentage of cancer pathway-related assays in all ToxCast platforms linked to characteristic, identified by IARC <sup>b</sup>
1. Electrophilic or ability to undergo metabolic activation	0% (0/100)	11% (31/274)
2. Genotoxic	4% (4/100)	3% (9/274)
3. Alter DNA repair or cause genomic instability	0% (0/100)	0% (0/274)
4. Epigenetic alterations	4% (4/100)	4% (11/274)
5. Oxidative stressor	4% (4/100)	7% (18/274)
6. Induce chronic inflammation	40% (40/100) <sup>c</sup>	16% (45/274)
7. Immunosuppressant	0% (0/100)	0% (0/274)
8. Modulate receptor-mediated effects	0% (0/100)	33% (92/274)
9. Immortalization	0% (0/100)	0% (0/274)
10. Alter cell proliferation, cell death, and nutrient supply	62% (62/100) <sup>d</sup>	25% (68/274)

<sup>a</sup>The 100 cancer pathway-related assays identified in this study were mapped to the 10 key characteristics of carcinogens described by IARC (IARC, 2015). Some of these assays were mapped to 2 characteristics.

<sup>b</sup>IARC mapped 274 cancer pathway-related assays from all 7 of the ToxCast platforms to the 10 key characteristics of carcinogens. They mapped each assay to 1 characteristic. Additional information can be found in the Excel file "Section 4.3 Spreadsheet" available at: <http://monographs.iarc.fr/NCI/Monographs/v112/>.

<sup>c</sup>Of the 40 assays mapped to induction of chronic inflammation, 14 evaluated altered cytokine production, 22 evaluated altered chemokine production, and 4 evaluated altered immunoglobulin production.

<sup>d</sup>Of the 62 assays mapped to altered cell proliferation, cell death, and nutrient supply, 18 evaluated increased cell proliferation, 2 evaluated decreased cell cycle arrest, 14 were associated with signaling pathways related to cellular replication/cell cycle control, and 28 were associated with angiogenesis.

• Most of the cancer pathway-related assays identified in this study evaluated induction of chronic inflammation and altered cell proliferation, cell death, and nutrient supply. A few of the assays evaluated genotoxicity, epigenetic alterations, and oxidative stress.

• None of the assays identified in this study evaluated electrophilicity or metabolic activation, altered DNA repair or genomic instability, immunosuppression, modulation of receptor-mediated effects, or immortalization.

• For comparison purposes, the percentages of assays from all the ToxCast platforms mapped to each key characteristic by IARC (IARC, 2015) are displayed here. The complete set of ToxCast assays includes some that evaluate electrophilicity or metabolic activation and modulation of receptor-mediated effects, which are not captured in the subset of assays we considered.

**Table 2.** Top 5% of chemicals active in cancer pathway-related assays.

1. Gentian violet	21. UK-337312 <sup>a</sup>	41. Clotrimazole
2. Octyl gallate	22. 2,4-Bis(2-methylbutan-2-yl)phenol	42. Diethylstilbestrol <sup>a</sup>
3. Didecylmethylammonium chloride	23. PharmaGSID_47315 <sup>a</sup>	43. 4-Octylphenol
4. Tamoxifen <sup>b</sup>	24. SB236057A <sup>a</sup>	44. Zoxamide
5. Triclosan	25. UK-373911 <sup>a</sup>	45. Clomiphene citrate <sup>a</sup>
6. Fluzinam	26. Thiram	46. Surinabant <sup>a</sup>
7. Tamoxifen citrate <sup>b</sup>	27. PharmaGSID_47337 <sup>a</sup>	47. Chlorothalonil
8. AVE5638 <sup>a</sup>	28. 4-(1,1,3,3-Tetramethylbutyl)phenol	48. Triphenyltin hydroxide
9. AVE8923 <sup>a</sup>	29. Captafol	49. Amiodarone hydrochloride <sup>a</sup>
10. Ocithilinine	30. 3-Iodo-2-propynyl-N-butylcarbamate	50. 3,3',5,5'-Tetrabromobisphenol A
11. SR146131 <sup>a</sup>	31. AVE6324 <sup>a</sup>	
12. 9-Phenanthrol <sup>a</sup>	32. 2,4-Di-tert-butylphenol	
13. Disulfiram <sup>a</sup>	33. PD 0343701 <sup>a</sup>	
14. Kepone	34. 4,4'-Sulfonylbis[2-(prop-2-en-1-yl)phenol]	
15. 1,2-Benzisothiazolin-3-one	35. SSR241586 <sup>a</sup>	
16. 4-Nonylphenol, branched	36. Dodecyltrimethylammonium chloride	
17. Tributyltin methacrylate	37. Ziram	
18. Phenylmercuric acetate	38. Farglitazar <sup>a</sup>	
19. Tributyltin chloride	39. Heptachlor	
20. SAR115740 <sup>a</sup>	40. 2,4-Bis(1-methyl-1-phenylethyl)phenol	

Chemicals shown in blue are listed under California's Proposition 65 as causing cancer (Title 27, California Code of Regulations, § 27001).

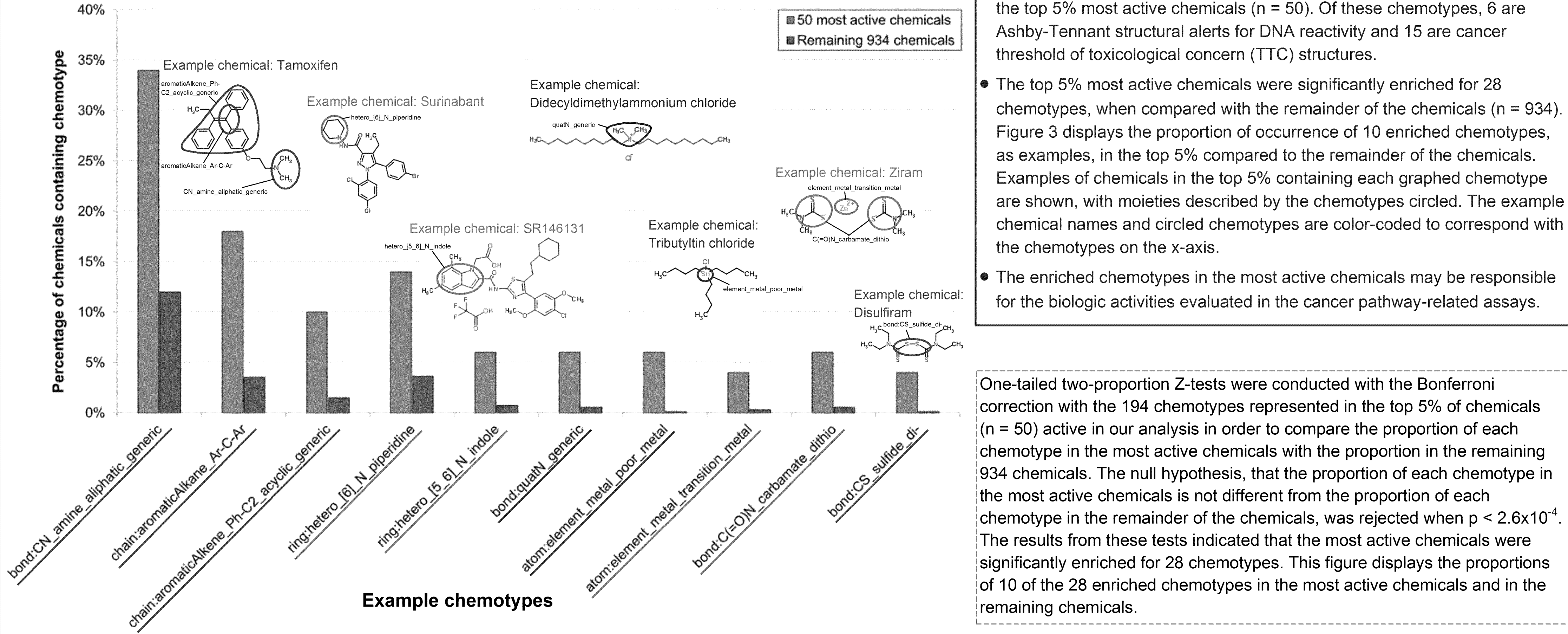
<sup>a</sup>Pharmaceutical compound.

<sup>b</sup>The citrate salt of tamoxifen; tamoxifen is listed under Proposition 65 as causing cancer.

• The top 5% most active chemicals (n = 50) were active in at least 40 (and up to 47) cancer pathway-related assays.

• Eight of these chemicals are listed under California's Proposition 65 as causing cancer (Title 27, California Code of Regulations, § 27001).

**Figure 3.** Chemicals most active in cancer pathway-related assays are enriched for specific chemotypes.



## Discussion and conclusions

ToxCast high-throughput assays that measure endpoints related to cancer pathways can be used to begin to identify and prioritize chemicals with possible carcinogenicity concern. Here, we identified cancer pathway-related assays and determined which ToxCast chemicals were the most active in this subset of assays, using an activity score that accounted for both quantity of assays and potency. An advantage to our approach is that it is easily modifiable. For example, the set of cancer-related assay endpoints could be expanded to capture more of the 10 key characteristics of carcinogens, which would improve the identification and prioritization of chemicals that may be of carcinogenic concern. We also identified enriched chemotypes in the most active chemicals. These chemotypes could be used to flag other chemicals as priorities for carcinogenicity and toxicity testing.

Linking high-throughput assays to the key biological events contributing to a toxicity endpoint, as we have begun to do in this study, is a promising way to quickly identify chemicals of possible health concern. Ultimately, this type of approach can help efficiently prioritize chemicals as candidates for health risk assessment.

## Acknowledgements

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